PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Shigeru YAMAMOTO, et al.

Docket No: Q63731

Appln. No.: 09/806,413

Group Art Unit: 1652

Confirmation No.: 8678

Examiner: David J. Steadman

Filed: March 30, 2001

For: NOVEL ENZYME COMPOSITION AND PRODUCTION METHOD AND USE THEREOF

SUBMISSION OF EXECUTED DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Submitted herewith is a copy of an executed Declaration Under 37 C.F.R. §1.132 signed by Kazutaka TSURUHAMI .

Respectfully submitted,

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Date: May 13, 2004

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DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Kazutaka TSURUHAMI hereby declare and state:

THAT I am one of the inventors of the invention disclosed and claimed in the above-identified application;

THAT I am a citizen of JAPAN;

THAT I received the degree of Master in1997 from Shimane University;

THAT I have been employed by Amano Enzyme Inc. since April 1 of 1993, where I hold a position as a researcher, with responsibility for research and development on enzymes, and their applications.

THAT I am fully familiar with the above-identified U.S. application (hereinafter referred to as "present invention" for brevity);

THAT I have read and am fully familiar with the art cited against claims of the above-identified U.S. application (hereinafter referred to as "present application" for brevity);

I SUBMIT herewith this Declaration in order to demonstrate that the above-identified application describes and enables diglycosidases from *Penicillium multicolor* microorganisms:

Object of experiment

The specification of the present application describes enzymological properties and molecular weight of the purified enzyme from *Aspergillus fumigates* and also describes production of other diglycosidases from microorganisms other than *Aspergillus fumigatus*. Taking diglycosidase derived from *Penicillium multicolor* in the genus *Penicillium* as an example, the enzyme was purified in accordance with the description in the present specification and the enzymological properties and the molecular weight of the purified enzyme were measured.

(1) Strain used

Penicillium multicolor IAM 7153

(2) Culturing method

Cultured in accordance with the method described from line 5 of page 40 to line 10 of page 41 of the present specification.

(3) Purification method

Purified in accordance with the method described from line 6 of page 46 to line 12 of page 47 to obtain a purified enzyme sample of diglycosidase. Purification was confirmed as a single band in SDS-PAGE.

(4) Physicochemical properties of diglycosidase

Physicochemical properties of the diglycosidase derived from *Penicillium multicolor* were measured in accordance with the methods described from line 13 of page 47 to line 16 of page 49. The results are shown in Table 1 below.

Table 1

Action and substrate specificity: Acts on β-primeverosidc to form β -primeverose and an aglycone.
Optimum pH: Around 4. Also shows activity at pH 2.5 to 3.
Optimum temperature: 55°C
Temperature stability: Stable at 50°C or lower

(5) Measurement of molecular weight

SDS-PAGE procedure was repeated for 4 times. Mobilities of the bands corresponding to the molecular weight markers and the object enzyme were measured and the results are shown in Table 2 below.

Table 2

	Mobility (cm)				
Molecular weight marker	KDa	No. 1	No. 2	No. 3	No. 4
Phosphorylase	97.4	2.10	1.73	1.88	1.90
Bovine albumin	66.3	2.88	2.30	2.48	2.58
Aldolase	42.4	4.45	3.53	3.98	3.95
Carbonic anhydrase	30.0	6.68	5.58	6.13	5.95
Trypsin inhibitor	20.1	8.58	7.40	7.98	7.73
Lysozyme	14.4	10.78	9.50	10.08	9.85
Enzyme sample		3.80	3.15	3.35	3.40

From the results in Table 2, molecular weights were determined and an average molecular weight was obtained. The results are shown in Table 3 below.

Table 3

	MW (kDA)					
	No. 1	No.2	No. 3	No. 4	Average MW	Standard Deviation (SD)
Enzyme Sample	52.0	50.8	52.1	51.7	51.7	0.6

Conclusion

The MW of the diglycosidase derived from the genus *Penicillium* has an approximate molecular weight of 49.9 kDa to 53.5 kDa (average MW $\pm 3\sigma$) as determined by SDS-PAGE.

From the above results, it was elucidated that the diglycosidase derived from the genus *Penicillium* has the same enzymological properties with those of the diglycosidase derived from *Asp., fumigatus* and the molecular weight is also similar between them.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: May 11, 2004

Kazutaka Tsuruhami
Kazutaka TSURUHAMI